# Abstract

Genus *Mycobacterium* comprises a large number of species including many pathogens such as *Mycobacterium leprae, Mycobacterium abscessus* and *Mycobacterium tuberculosis* (*Mtb*), the last one is the causative agent of the fatal disease tuberculosis. The unique features of the deadly organism *viz,* slow growing, tough cell wall, latency and resistance to various drugs demand a systematic understanding of many essential molecular processes including transcription. Studies have been undertaken to understand several aspects of transcription in mycobacteria which revealed its machinery to be conserved with other eubacteria? However, many facets of transcription in mycobacteria and regulation are different. The transcription regulators and them regulation are the basic counter stones which govern gene expression. The present study is aimed to understand better the mechanistic regulation of transcription of important housekeeping functions, DNA gyrase and also to obtain further insights into the role of transcription elongation factor Gre.

**Chapter 1** of the thesis provides a general introduction of the bacterial transcription machinery, associated transcription regulators and their regulation. It covers the description of the central player- the RNA polymerase (RNAP) followed by each step of the transcription initiation, elongation and various factors involved in their regulation. Finally, an overview of the emerging information on several aspects of mycobacterial transcription is discussed emphasizing on RNAP, promoter architecture, and its regulation.

**In Chapter 2**, the studies are directed to understand the mechanism for topology-dependent regulation of *Mtb* Gyrase. The gyrase is encoded by two genes *gyrB* and *gyrA* which form a bicistronicity operon in *Mtb* and harbor multiple promoters. The principal promoter P*gyrB1* drives the transcription of the dicistron and the weaker divergent promoter P*gyrR* is engaged in transcription in the opposite direction. The divergent and overlapping P*gyrR* show decrease in activity when the P*gyrB1* was induced upon relaxation of the genome by a phenomenon termed relaxation stimulated transcription (RST). P*gyrR* plays a role in the fine tuning of *gyr* gene expression by reiterative transcription (RT), a regulatory mechanism hitherto not described in *Mtb*. *In vitro* transcription assays show that RT at P*gyrR* is dependent on the negatively supercoiled status of the DNA. The principal promoter P*gyrB1* is also regulated by DNA topology but does not exhibit RT. It is elucidated that the RNAP binding is efficient at P*gyrB1* when the DNA is relaxed whereas binding to P*gyrR* is preferred when DNA is supercoiled. Thus, a collaboration between RST and RT govern the regulation of *gyr* operon; the differential topology sensitivity of the overlapping promoters determines and dictate the efficiency of transcription initiation at *gyr* promoters. In addition, this study suggests a new mechanism of RST distinct from the one observed for other bacteria, such as *E. coli* or *M. smegmatis.*

**Chapter 3** describes studies that have been carried out to delineate the mechanism underlying the differential function of transcription regulator *Mtb*GreA and its homolog Rv3788 (*Mtb*Gfh1). *Mtb*GreA binds to RNAP and induces the intrinsic transcript cleavage activity of RNAP thereby allowing RNAP to resume transcription from paused and arrested sites. In spite of having Gre like domains, *Mtb*Gfh1 does not stimulate RNA cleavage. Instead, it inhibits transcription by binding to RNAP. Homology modeling and docking data suggest that Gre and *Mtb*Gfh1 bind to RNAP in a different orientation. *Mtb*GreA coordinate with the Mg2+ present in the catalytic center of the RNAP while *Mtb*Gfh1 was observed to be facing away from Mg2+ Swapping of a stretch of residues from the N-terminus of *Mtb*GreA into *Mtb*Gfh1 acquire GreA like transcript cleavage stimulatory activity and enhance promoter clearance for *Mtb*Gfh1. Bioinformatics analysis and biochemical assays demonstrate the significance of a stretch of residues in the N-terminus of *Mtb*GreA and *Mtb*Gfh1 for their functions. Also, the orientation of the *Mtb*GreA and *Mtb*Gfh1 while binding to RNAP is a crucial determinant in governing their respective function. Being the general inhibitor of transcription, overexpression of *Mtb*Gfh1 led to the appearance of tiny colonies and slow growth of cells suggesting its regulatory role to maintain the physiology of *Mtb*.

In **Chapter 4,** the influence of perturbation of GreA level on *Mtb* growth and physiology has been studied. *Mtb* contains a single Gre protein (Rv1080c), unlike many other bacteria where both GreA and GreB are present. Further, the GC-rich genome of *Mtb* may pose an additional challenge to the transcribing RNAP. Hence the role of GreA could be essential to maintain high fidelity of transcription and RNAP distribution in *Mtb* genome. To validate this, the conditional knockdown strain of *Mtb*GreA was generated. GreA depleted strain exhibited slow growth and caused phenotypical changes in *Mtb* cells. Moreover, the occupancy of RNAP on the promoter and gene body of candidate gene tested was found to be disrupted upon *Mtb*GreA depletion, suggesting the regulatory role of GreA in modulating *Mtb* physiology.